Refine Search

Search Results -

Terms	Documents
(amyloidosis or alzheimer$) and ($estradiol) and ($dihydroequilenis or premarin$)	78

Database:

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US Pre-Grant Publication Full-Text Database
US Patents Full-Text Database
US OCR Full-Text Database
EPO Abstracts Database
JPO Abstracts Database
Derwent World Patents Index
IBM Technical Disclosure Bulletins
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Search: L2

[Refine Search]

[Recall Text] [Clear] [Interrupt]

Search History

DATE: **Tuesday, February 28, 2006**   Printable Copy   Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR			
L2	(amyloidosis or alzheimer$) and ($estradiol) and ($dihydroequilenis or premarin$)	78	L2
L1	(amyloidosis or alzheimer$) same ($estradiol) same ($dihydroequilenis or premarin$)	0	L1

END OF SEARCH HISTORY

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L2: Entry 14 of 78   File: USPT   Apr 26, 2005

DOCUMENT-IDENTIFIER: US 6884795 B2
TITLE: Pharmaceutical compositions and uses for androst-5-ene-3.beta., 17.beta.-diol

Brief Summary Text (5):
5-DIOL is a compound biosynthesized from DHEA through the action of reductive
17.beta.-hydroxysteroid dehydrogenase (17.beta.-HSD) and is a weak estrogen. It has
an 85-fold lower affinity than 17.beta.-estradiol (E.sub.2) for the estrogen
receptor in rat anterior pituitary gland cytosol (Simard and Labrie, J. Steroid
Biochem., 26: 539-546, 1987), further confirming the data obtained on the same
parameter in human myometrial and breast cancer tissue (Kreitmann and Bayard, J.
Steroid Biochem., 11: 1589-1595, 1979; Adams et al., Cancer Res., 41: 4720-4926,
1981; Poulin and Labrie, Cancer Res., 46: 4933-4937, 1986).

Brief Summary Text (10):
In addition, breast cancer, cardiovascular disease, and insulin resistance have
been associated with decreased serum levels of DHEA and DHEA-S and both DHEA and
DHEA-S have been suggested to prevent or treat these conditions. DHEA has also been
suggested to have beneficial effects in the treatment and/or prevention of obesity,
diabetes, atherosclerosis, chemically-induced breast, skin, and colon cancer,
autoimmune diseases, Alzheimer's disease, loss of memory, aging and to support
energy, muscle mass, and longevity. Uses of DHEA as well as the benefits of
androgen and estrogen therapy are discussed in International Patent Publication WO
94/16709.

Brief Summary Text (15):
It is a further object of this invention to provide methods of or treating or
reducing the risk of onset of conditions which respond favorably to estrogenic
activity, including vaginal atrophy, hypogonadism, diminished libido, skin atrophy,
urinary incontinence, lipid, and lipoprotein imbalance, atherosclerosis,
cardiovascular disease and symptoms of menopause (hot flushes, sleep disorders,
Alzheimer's disease, Parkinson's disease, mental disorders, depression, loss of
memory) by administering 5-DIOL. It is a further object of this invention to
provide methods of preventing or treating conditions which respond favorably to
androgenic activity, including breast cancer, ovarian cancer, endometrial cancer,
diminished libido, skin atrophy, skin dryness, osteoporosis and symptoms of
menopause by administering 5-DIOL. A number of diseases that are affected by sex
steroids (e.g. osteoporosis) respond favorably to both androgens and estrogens.

Detailed Description Text (9):
Applicant has discovered that 5-DIOL produces significantly different androgenic
and estrogenic effects than does DHEA. In particular, 5-DIOL is shown to produce
less potential androgenic or masculinizing effects for a given production of
estrogenic effects than does DHEA. Therefore, 5-DIOL is particularly beneficial in
treating conditions which require estrogenic activity with minimal androgenic
activity. In fact, after menopause, women have a deficit of both androgens and
estrogens although the ratio of estrogen/androgen is lower than before menopause.
Women thus require a more favorable estrogenic-androgenic ratio to compensate the
loss of estradiol secretion by the ovaries. DHEA cannot compensate for this ovarian
estrogenic deficit but will only replace the lowered secretion of DHEA-S and DHEA

by the adrenals.

Detailed Description Text (18):
Individuals who will benefit from treatment with 5-DIOL include all these suffering
from conditions treatable with DHEA, including those with abnormally low levels of
5-DIOL, estrogen, or androgen. Reducing the risk of acquiring such conditions is
also possible and the recommended 5-DIOL dosage and target serum levels is the same
as for the therapeutic uses of 5-DIOL herein. Individuals who could benefit from
the invention can be identified by measuring serum levels of 5-DIOL, DHEA, sex
steroids and their metabolites (especially androsterone glucuronide and adrostrane-
3a, 17.beta.-diol glucuronide for androgens and estrogen-sulfate and estradiol-
sulfate for estrogens) as described by Belanger et al., in Steroid Formation,
Degradation and Action in Peripheral, Normal and Neoplastic tissues (H. Bradlow, L.
Castagnetta, S. d'Aquino, L. Gogliotti, eds) Ann. N.Y. Acad. Sci. 586: 93-100,
1990. Serum IGF-1 levels can be measured at described (Furlanetto et al., J. Clin.
Invest. 60:648, 1977).

Detailed Description Text (20):
In some preferred embodiments, serum concentration is between 4.0 and 7.0 or
between 7.0 and 15 nM for women and men, respectively. However, for purpose of
contraception or for prevention of ovarian or uterine cancer, concentrations up to
15 nM (e.g. between 10 and 13) may be preferred for women. For contraception, an
estrogen may be added (e.g. estradiol giving serum estradiol levels between 50 and
200 nanograms per liter), and an added progestin may be particularly appropriate.
Preferred dosages discussed herein may be increased as appropriate to achieve
desired serum concentrations, e.g. with variations for individuals patient response
as monitored by attending clinician.

Detailed Description Text (60):
Since 5-DIOL is converted to estrogen in many tissues, it is unlikely that estrogen
will need to be added to the contraceptive therapy to compensate for the decreased
estrogen production in the ovaries. However, minimum doses can be given, if
necessary. Preferred dosage of added estrogen, when used in the contraceptive
method is an amount effective to achieve between 50 and 300 nanograms estradiol per
liter or equivalent. Preferably the ratio of added estradiol to 5-DIOL (w/w) will
range from 100:1 to 10,000:1, preferably, 200:1 to 5,000:1 and especially 300:1 to
3000:1. As with added progestin, added estrogen may be administered as part of a
pharmaceutical composition that includes the 5-DIOL (or, where used, a prodrug of
5-DIOL) or separately.

Detailed Description Text (68):
However, if it is determined that additional estrogens are needed, the estrogen and
5-DIOL may be administered simultaneously or separately. In addition, it is
necessary only that both the 5-DIOL and estrogen by administered in a manner and at
a dosage sufficient to allow blood serum concentration of each to obtain desired
levels. In accordance with the combination therapy of the invention, concentration
is maintained within desired parameters at the same time that estrogen
concentration is maintained within desired parameters. Where estradiol is used,
serum estradiol concentration should typically be maintained between 50 and 200
nanograms per liter, preferably between 100 and 175 nanograms per liter and most
preferably between 125 and 175 nanograms per liter. Where another estrogen is used,
serum concentration may be varied in a known manner to account for the known
difference in estrogenic activity relative to estradiol and in order to achieve
normal premenopausal estrogen levels. A lesser concentration is needed, for
example, if Mestranol is used. Adequate serum estrogen levels can also be assessed
by disappearance of the symptoms of menopause. Serum concentration of the 5-DIOL is
typically maintained between 4 and 10 nM for women and between 10 and 20 nM for men
or in some embodiments between 4.0 and 7.0 nM for women or between 7.0 and 15 nM
for men.

Detailed Description Text (69):
If estrogen is combined with 5-DIOL, it is preferably estradiol, but may be
estrogen sulfate or another compound which acts as an estrogen receptor agonists
directly or following proper conversion. When administered separately, commercially
available estrogen supplements may be used, e.g., PREMARIN, available from Ayerst
(St. Laurent. Quebec, Canada). For typical patients, the appropriate dosage of
estrogen to achieve desired serum concentrations is between 0.3 and 2.5 milligrams
of PREMARIN per day per 50 kg of body weight when administered orally. In certain
embodiments of the invention, the estrogen may be 17.beta.-estradiol administered
percutaneously in a patch which is available from CIBA under the name ESTRADERM
wherein the daily doses is between 0.05 and 0.2 milligrams per day per 50 kg of
body weight. For typical patients, the appropriate dosage of the sex steroid
precursor 5-DIOL to achieve desired serum concentration of the precursor is between
0.10 and 2.5 grams per day per 50 kg of body weight when administered orally. Other
prodrugs will be administered at a dosage that depends on their in vivo conversion
rate to 5-DIOL. 5-DIOL may also be administered transdermally or transmucosally, as
described in detail above, in a sufficient amount to achieve target serum
concentration.

Other Reference Publication (42):
Boccuzzi G., et al., "5-ene-androsterone-3beta, 17beta-diol inhibits the growth of
MCF-7 breast cancer cells when estrogen receptors are blocked by estradiol", Br J
Cancer, Dec. 1994; 70(6):1035-9.

Other Reference Publication (43):
Boccuzzi G., et al., "Influence of dehydroepiandrosterone and 5-ene-androsterone-
3beta, 17beta-diol on the growth of MCF-7 human breast cancer cells induced by
17beta-estradiol" Anticancer Res, May-Jun. 1992; 12(3):799-803.

L2: Entry 18 of 78   File: USPT   Feb 15, 2005

DOCUMENT-IDENTIFIER: US 6855704 B2
TITLE: Process for isolating conjugated estrogens

Brief Summary Text (4):
Menopause is generally defined as the last natural menstrual period and is
characterized by the cessation of ovarian function, leading to the substantial
diminution of circulating estrogen in the bloodstream. Menopause is usually
identified, in retrospect, after 12 months of amenorrhea. It is not a sudden event,
but is often preceded by a time of irregular menstrual cycles prior to eventual
cessation of menses. Following the cessation of menstruation the decline in
endogenous estrogen concentrations is typically rapid. There is a decrease in serum
estrogens from circulating levels ranging from 40-250 pg/mL of estradiol and 40-170
pg/ml of estrone during ovulatory cycles to less than 15 pg/mL of estradiol and 30
pg/mL of estrone in postmenopausal women.

Brief Summary Text (6):
Estrogen replacement therapy (ERT) is beneficial for symptomatic relief of hot
flushes and genital atrophy and for prevention of postmenopausal osteoporosis. ERT
has been recognized as an advantageous treatment for relief of vasomotor symptoms.
There is no acceptable alternative to estrogen treatment for the atrophic changes
in the vagina; estrogen therapy increases the vaginal mucosa and decreases vaginal
dryness. Long term ERT is the key to preventing osteoporosis because it decreases
bone loss, reduces spine and hip fracture, and prevents loss of height. In
addition, ERT has been shown to be effective in increasing high density
Lipoprotein-cholesterol (HDL-C) and in reducing low density lipoprotein cholesterol
(LDL-C), affording possible protection against CHD. ERT also can provide
antioxidant protection against free radical mediated disorders or disease states.
Estrogens have also been reported to confer neuroprotection, and inhibit
neurodegenerative disorders, such as Alzheimer's disease. The best known oral
estrogen replacement therapy available in the United States is a natural mixture of
conjugated equine estrogens sold under the trademark Premarin.

Detailed Description Text (3):
Conjugated estrogens as described in the United States Pharmacopeia 23, National
Formulary 18, Official January, 1995 (USP23) is a mixture of sodium estrogen
sulfated and sodium equilin sulfate, derived wholly or in part from equine urine or
synthetically from estrone and equilin. The most abundant of the estrogens in the
standardized blend are the sulfate esters of estrone and equilin. It contains other
conjugated estrogenic substances of the type excreted by pregnant mares.
Concomitant to the sodium sulfate esters of estrone and equilin are the compounds
17 .alpha.-dihydroequilin, 17 .beta.-dihydroequilin and 17 .alpha.-estradiol.
Signal impurities derived from degradation of the equilin are 17.alpha.
dihydroequilenin, 17 .beta.-dihydroequilenin and equilenin. Other sodium sulfate
esters of steroids that may be present in conjugated estrogens are 17 .beta.-
estradiol and D.sup.8,9 -dehydroestrone. Sodium sulfate esters of estrone, equilin
and the concomitant components are required by USP23 to present in all dosage forms
of conjugated estrogen. These compounds are subject to an upper and lower limit of
their concentration.

Detailed Description Text (4):

One example of a conjugated estrogen product for use in estrogen replacement therapy is Premarin (conjugated estrogens tables, (USP) for oral administration that contains a mixture of estrogens obtained exclusively from natural sources, occurring as the sodium salts of water-soluble estrogen sulfated blended to represent the average composition of material derived from pregnant mares' urine. It is a mixture of sodium estrone sulfate and sodium equilin sulfate, and at least the following 8 concomitant components, also as sodium sulfate conjugated: 17 .alpha.-dihydroequili, 17 .alpha.-estradiol, D.sup.8,9 -dehydroestrone, 17 .beta.-dihydroequilin, 17 .beta.-estradiol, equilenin, 17 .alpha.-dihydroequilenin, and 17 .beta.-dihydroequilenin. The make up of Premarin conjugated estrogens is currently being analyzed, and other components are in the process of being identified and characterized. See, for example, Baracat et al (1999) and Dey et al (2000). Premarin conjugated estrogens is indicted in the treatment of moderate to severe vasomotor symptoms associated with the menopause; treatment of vulvar and vaginal atrophy; and prevention of osteoporosis, as well as other indications approved for estrogen products.

Detailed Description Text (28):
In a second aspect, the present invention relates to a process for obtaining a natural mixture of conjugated estrogens. The conjugated estrogen product may meet the requirements of USP23, or it may meet the requirements of the FDA Guidance for Industry Conjugated Estrogens, USP-LC, MS Method for Quantitative Chemical Characterization and Documentation of Qualitative Pharmaceutical Equivalence, dated March 2000 ("FDA Guidance"), or it may meet the component profile of the Premarin conjugate estrogens as determined by gas and liquid chromatographic techniques (FDA Guidance). In accordance with this aspect of the invention, sub-batches of the major and minor estrogen components are prepared as described above from different urine shipments, each of which is harvested at different intervals during a gestation period of the mares. Each sub-batch is analyzed for individual compounds using conventional techniques, and the data is stored in a spread sheet. From the analysis of this collective data, a blending procedure is performed to produce a natural component mixed active ingredient which meets the requirements of USP23, the FDA Guidance or the component profile of the Premarin conjugate estrogens.

Detailed Description Text (63):
Several such iterations/sub-batches of the major and minor components were prepared from different urine shipments, each of which was harvested at different intervals during the gestation period of the mares. Each such sub-batch was analyzed for individual compounds and the data was stored in a spread sheet. From evaluation of this collective data, blending procedure was initiated to produce a natural components mixed active ingredient which mimics the component profile of the reference drug product (e.g., Premarin conjugated estrogens) was determined via gas and liquid chromatographic techniques in accordance with FDA guidance for Industry "Conjugated Estrogens, USP Ly LC, LC-MS Method for quantitative Chemical Characterization and Documentation of Qualitative Pharmaceutical Equivalence, dated March 200 (FDA Guidance).

Detailed Description Text (64):
LC/MS analysis of a lot of Premarin conjugated estrogens (lot #A08775) and a conjugated estrogen drug substance prepared above were analyzed in accordance with the FDA Guidance. The FDA Guidance requires injection of conjugated estrogen standard. Unfortunately, the USP sells only the unconjugated estrogen standards. Therefore, it was decided with analysis, i.e, obtaining relative areas, would be preformed by quantitating against the three major conjugated estrogen components present in each of the samples and run.

Detailed Description Text (67):
From the plot of m/z versus the relative area % for peaks greater than 1%, both the products exhibit 21 major m/z. However, the lot according to the present example has seven peaks that are not present in the Premarin lot and the Premarin lot has

five peaks that are not present in the lot of the present example. In the case of the Premarin lot's five peaks with area greater than 1% eluting at an RRT of about 0.08 were excluded on the assumption that they are due to excipients in the brand formulation.

Detailed Description Text (68):
In view of this data, it is believed that, based upon the FDA Guidance, a Conjugated Estrogens API prepared in accordance with the present invention should be considered equivalent to or the "same as" Premarin conjugated estrogens API for the purposes of utilization in the development of a therapeutically equivalent generic conjugated estrogens drug product.

Detailed Description Paragraph Table (2):
TABLE 2 Summary of Peaks Observed for Relative Area % vs. RRT Number of Peaks Number of Number of Peaks Number of Peaks Recalculated Observed Peaks Common with Different from RRT Based on Decimal % Relative Area (raw data) Other Source Other Source Decimal Place Place Round Off Example Lot > 1% 44 29 15 3 42 Premarin .RTM. Lot > 1% 33 25 8 3 32 Example Lot > 0.1% 216 4 87 but < 1% Premarin .RTM. Lot > 190 4 84 0.1% but < 1%

L2: Entry 41 of 78   File: USPT   Jul 30, 2002

DOCUMENT-IDENTIFIER: US 6426097 B2
TITLE: Herbal supplement for cognitive related impairment due to estrogen loss

Brief Summary Text (4):
Menopause is a condition in women wherein their bodies no longer produce estrogen
and is responsible for physical and physiological changes in the body including
bone loss, osteoporosis, cardiovascular disease, senile dementia/Alzheimer's
disease, flushing (hotflashes), urogenital atrophy, dysmenorrhea and acne.
Decreased production of estrogen, whether from normal onset of menopause or
surgically induced menopause through the removal of the ovaries, is also associated
with impaired cognitive function, particularly memory and attention loss, and
neurodegeneration.

Brief Summary Text (9):
Acetylcholinesterase inhibitors enhance the effects of acetycholine, either by
inhibiting its hydrolyzation or increasing the time the acetylcholine is present in
the synapse. Galanthamine is a known acetylcholinesterase inhibitor. Galanthamine
reversibly binds to acetylcholinesterase, inhibiting its action and resulting in an
increase in local concentrations of acetylcholine. Galanthamine has been used in
the treatment of different diseases of the nervous system such as Alzheimer's
disease (U.S. Pat. No. 5,958,903) and Parkinson's disease (5,965,571); the
treatment of chronic fatigue syndrome (5,312,817); as an erectogenic agent in the
treatment of male sexual dysfunction (5,177,070) as well as the treatment of
glaucoma, myasthenia gravis and senile dementia.

Brief Summary Text (11):
A variety of hormone replacement therapies are presently available to help
alleviate the deleterious physical and physiological changes associated with
menopause. Such regimens include combination therapies using estradiol and
conjugated equine estrogens such as Premarin.RTM. (Wyeth-Ayerst Laboratories,
Princeton, N.J.). Hormone replacement therapies provide benefits in the areas of
cardiovascular disease and bone loss, however have numerous side effects including
endometrial cancer, increased risk of breast cancer, and vaginal and uterine
bleeding. Single agent hormone replacement therapy using the steroidal compound 17
alpha-dihydroequilenin has been disclosed to prevent neurodegeneration associated
with cognitive dysfunction in estrogen deficient conditions including menopause
(U.S. Pat. No. 5,719,137).

Other Reference Publication (1):
Shua-Haim, J.R., et al., "Current and The Near Future Medications for Alzheimer's
disease: What can we Expect from Them"?American Journal of Alzhemiers Disesase,
vol. 14, No. 5, Sep./Oct. (1999), pp. 294-307.

Other Reference Publication (2):
Peskind, Elaine R., M.D., "Pharmacologic Approaches to Cognitive Deficits in
Alzheimer's Disease", J. Clin. Psychiatry, vol. 59 (suppl.), No. 9, (1998), pp. 22-
27.

L2: Entry 50 of 78   File: USPT   Dec 12, 2000

DOCUMENT-IDENTIFIER: US 6159959 A
TITLE: Combined estrogen and antiestrogen therapy

Brief Summary Text (3):
The use of hormone replacement therapy for bone loss prevention in post-menopausal
women is well precedented. The normal protocol calls for estrogen supplementation
using such formulations containing estrone, estriol, ethynyl estradiol or
conjugated estrogens isolated from natural sources (i.e. Premarin.RTM. conjugated
estrogens from Wyeth-Ayerst). In some patients, therapy may be contraindicated due
to the proliferative effects of unopposed estrogens (estrogens not given in
combination with progestins) have on uterine tissue. This proliferation is
associated with increased risk for endometriosis and/or endometrial cancer. The
effects of unopposed estrogens on breast tissue is less clear, but is of some
concern. The need for estrogens which can maintain the bone sparing effect while
minimizing the proliferative effects in the uterus and breast is evident. Certain
nonsteroidal antiestrogens have been shown to maintain bone mass in the
ovariectomized rat model as well as in human clinical trials. Tamoxifen (sold as
Novadex.RTM. brand tamoxifen citrate by Zeneca Pharmaceuticals, Wilmington, Del.),
for example, is a useful palliative for the treatment of breast cancer and has been
demonstrated to exert an estrogen agonist-like effect on the bone, in humans.
However, it is also a partial agonist in the uterus and this is cause for some
concern. Raloxifene, a benzothiophene antiestrogen, has been shown to stimulate
uterine growth in the ovariectomized rat to a lesser extent than Tamoxifen while
maintaining the ability to spare bone. A suitable review of tissue selective
estrogens is seen in the article "Tissue-Selective Actions Of Estrogen Analogs",
Bone Vol. 17, No. 4, October 1995, 181S-190S.

Brief Summary Text (11):
Compounds of the general structure type shown in formulas (I) and (II) are estrogen
agonists/antagonists useful for the treatment of diseases associated with estrogen
deficiency. The compounds of the present invention show strong binding to the
estrogen receptor. These compounds have proven to be antiestrogens with little
intrinsic estrogenicity. The compounds of formula (I) are capable of antagonizing
the effects of 17.beta.-estradiol while showing little uterine stimulation when
dosed alone.

Brief Summary Text (36):
Estrogens useful in the formulations of this invention include estrone, equilin,
equilenin, ethynyl estradiol, 17.beta.-estradiol, dihydroequilenin, 17.beta.-
dihydroequilenin (U.S. Pat. No. 2,834,712), menstranol and conjugated estrogenic
hormones, such as those in Wyeth-Ayerst Laboratories' Premarin.RTM. products, as
well as the sulfated esters of these estrogens. Also useful in the present
formulations are Sodium estrone sulfate, Sodium equilin sulfate, Sodium 17alpha-
dihydroequilin sulfate, Sodium 17alpha-estradiol sulfate, Sodium Delta8,9-
dehydroestrone sulfate, Sodium equilenin sulfate, Sodium 17beta-dihydroequilin
sulfate, Sodium 17alpha-dihydroequilenin sulfate, Sodium 17beta-estradiol sulfate,
Sodium 17beta-dihydroequilenin sulfate, Estrone 3-sodium sulfate, Equilin 3-sodium
sulfate, 17alpha-Dihydroequilin 3-sodium sulfate, 3beta-Hydroxy-estra-5(10),7-dien-
17-one 3-sodium sulfate, 5alpha-Pregnan-3beta-20R-diol 20-sodium sulfate, 5alpha-
Pregnan-3beta,16alpha-diol,20-one 3-sodium sulfate, delta(8,9)-Dehydroestrone 3-

sodium sulfate, Estra-3beta,17alpha-diol 3-sodium sulfate, 3beta-Hydroxy-estr-5
(10)-en,17-one 3-sodium sulfate or 5alpha-Pregnan-3beta, 16alpha,20R-triol 3-sodium
sulfate. Esterified estrogens, such as those sold by Solvay Pharmaceuticals, Inc.
under the Estratab.RTM. tradename, may also be used with the present formulations.
Preferred salts of estrone include, but are not limited to, the sodium and piperate
salts. Phytoestrogens, such as equol or enterolactone, may also be used in the
present formulations and methods. Mammalian metabolic conjugates of estrogens
hereunder, such as the sulfates or glucoronides thereof, may be preferred. A
particularly preferred embodiment of this invention comprises pharmaceutical
compositions and methods of treatment utilizing conjugated estrogenic hormones,
such as those in Wyeth-Ayerst Laboratories' Premarin.RTM. products, with one or
more compounds of Formulas (I) or (III) listed herein.

Brief Summary Text (37):
The present compounds of Formulas (I) and (II) are tissue selective compounds
having the ability to behave like estrogen agonists, such as by lowering
cholesterol and preventing bone loss, or like estrogen antagonists. Therefore,
these compounds in the present formulations are useful for treating many maladies
including osteoporosis, prostatic hypertrophy, infertility, breast cancer,
endometrial hyperplasia, endometrial cancer, endometriosis, cystic glandular
hyperplasia, uterine hyperplasia, cervical hyperplasia, benign prostatic
hyperplasia, cardiovascular disease, contraception, Alzheimer's disease and
melanoma. The formulations of this invention may also be used to treat bone loss
resulting from secondary osteoporosis, including that categorized as endocrine in
nature, including that resulting from glucocorticoid excess, hyperparathyroidism,
hyperthyroidism, hypogonadism, hyperprolactinemia, and diabetes mellitus. The bone
loss may also be the drug-induced, such as that resulting from heparin treatments,
alcohol consumption, or the use of tobacco, barbiturates or corticosteroids. The
drug-induced loss of bone may also stem from treatment with gonadotropin releasing
hormone (GnRH or LHRH) or synthetic GnRH antagonists or agonists, such as the
leuprolide acetate injectable sold by TAP Pharmaceuticals Inc. under the tradename
LUPRON.RTM. or the goserelin acetate implant sold by Zeneca Pharmaceuticals under
the Zoladex.RTM. tradename. Such bone loss may also result from immobilization of
the individual, chronic renal failure, malabsorption syndrome, hepatic disease,
chronic obstructive lung disease, rheumatoid arthritis, or sarcoidosis.

Brief Summary Text (47):
It will be understood that the estrogen of this invention will be administered in
the dosages of conventional regimens, according to the recipient's tolerance and
the particular treatment or maintenance schedule intended. The compounds of
Formulas (I) and (II) herein will be administered in an amount necessary to agonize
or antagonize the estrogen(s) of the formulation's activity to the level desired.
When conjugated estrogens, USP, are used, it is preferred that the daily doseage is
from 0.3 mg to 5.0 mg, more preferably between about 0.3 mg and about 2.5 mg, most
preferably between about 0.3 and about 1.25 mg/day. For mestranol a daily dosage
may be from about 1 .mu.g to about 0.15 mg/day and a dosage of from about 1 .mu.g
to about 0.03 mg/day may be used for ethynyl estradiol, preferably between about
5 .mu.g to about 0.15 mg/day of ethynyl estradiol.

Detailed Description Text (94):
The competition assay was performed in a 96-well plate (polystyrene*) which binds
<2.0% of the total input [.sup.3 H]-17.beta.-estradiol and each data point was
gathered in triplicate. 100uG/100 uL of the receptor preparation was aliquoted per
well. A saturating dose of 2.5 nM [.sup.3 H]17.beta.-estradiol+competitor (or
buffer) in a 50 uL volume was added in the preliminary competition when 100.times.
and 500.times. competitor were evaluated, only 0.8 nM [.sup.3 H] 17.beta.-estradiol
was used. The plate was incubated at room temperature for 2.5 h. At the end of this
incubation period 150 uL of ice-cold dextran coated charcoal (5% activated charcoal
coated with 0.05% 69K dextran) was added to each well and the plate was immediately
centrifuged at 99 g for 5 minutes at 4.degree. C. 200 uL of the supernatant

solution was then removed for scintillation counting. Samples were counted to 2% or 10 minutes, whichever occurs first. Because polystyrene absorbs a small amount of [.sup.3 H] 17.beta.estradiol, wells containing radioactivity and cytosol, but not processed with charcoal were included to quantitate amounts of available isotope. Also, wells containing radioactivity but no cytosol were processed with charcoal to estimate unremovable DPM of [.sup.3 H] 17.beta.-estradiol.

Detailed Description Text (96):
Counts per minute (CPM) of radioactivity were automatically converted to disintegrated per minute (DPM) by the Beckman LS 7500 Scintillation Counter using a set of quenched standards to generate a H# for each sample. To calculate the % of estradiol binding in the presence of 100 or fold 500 fold competitor the following formula was applied:

Detailed Description Text (107):
The DNA (20 uG) was dissolved in 500 uL of 250 mM sterile CaCl.sub.2 and added dropwise to 500 uL of 2.times.HeBS (0.28 M NaCl, 50 mM HEPES, 1.5 mM Na.sub.2 HPO.sub.4, pH 7.05) and incubated at room temperature for 20 minutes. 20 uL of this mixture was added to each well of cells and remained on the cells for 16 h. At the end of this incubation the precipitate was removed, the cells were washed with media, fresh treatment media was replaced and the cells were treated with either vehicle, 1 nM 17.beta.-estradiol, 1 uM compound or 1 uM compound+1 nM 17.beta.-estradiol (tests for estrogen antagonism). Each treatment condition was performed on 8 wells (n=8) which were incubated for 24 h prior to the luciferase assay.

Detailed Description Text (115):
The luciferase data was generated as relative light units (RLUs) accumulated during a 10 second measurement and automatically transferred to a JMP (SAS Inc) file where background RLUs were subtracted. The B-galactosidase values were automatically imported into the file and these values were divided into the RLUs to normalize the data. The mean and standard deviations were determined from a n=8 for each treatment. Compounds activity was compared to 17.beta.-estradiol for each plate. Percentage of activity as compared to 17.beta.-estradiol was calculated using the formula %=((Estradiol-control)/(compound value)).times.100.

Detailed Description Text (116):
As can be seen from the data in Table 2, the benzo[a]carbazoles (#21 and #22) bind better to the ER receptor than the indenoindole #6. From the infection luciferase assay data in Table 3, it can be seen that none of the compounds show significant agonistic activity on this promoter. Benzo[a]carbazoles show an ability to antagonize the effects of estradiol to baseline or almost baseline levels.

Detailed Description Paragraph Equation (1):
((DPM sample-DPM not removed by charcoal/(DPM estradiol-DPM not removed by charcoal)).times.100%=% of estradiol binding

Detailed Description Paragraph Table (2):
	Compound % Activation
	17.beta.-estradiol 100% activity estriol 38%
activity Raloxifene 0%	

Detailed Description Paragraph Table (3):
TABLE 3 _____ Infection Luciferase Activity
##STR20## Compound # n Z 1 uM 1 uM + 17.beta. estradiol
_____ Cmpd. 6 (Scheme 2) 1 ##STR21## -2 83 Cmpd. 21 2(Scheme 4) ##STR22## -7 1 Cmpd. 22 2(Scheme 4) ##STR23## 1 8

CLAIMS:

16. A pharmaceutical composition of claim 1 wherein the one or more estrogens are selected from equilin, equilenin, ethinyl estradiol, 17.beta.-estradiol, dihydroequilenin, 17.beta.-dihydroequilenin, menstranol, conjugated estrogens, sulfate esters of estrone, Sodium estrone sulfate, Sodium equilin sulfate, Sodium 17alpha-dihydroequilin sulfate, Sodium 17alpha-estradiol sulfate, Sodium Delta8,9-dehydroestrone sulfate, Sodium equilenin sulfate, Sodium 17beta-dihydroequilin sulfate, Sodium 17alpha-dihydroequilenin sulfate, Sodium 17beta-estradiol sulfate, Sodium 17beta-dihydroequilenin sulfate, Estrone 3-sodium sulfate, Equilin 3-sodium sulfate, 17alpha-Dihydroequilin 3-sodium sulfate, 3beta-Hydroxy-estra-5(10), 7-dien-17-one 3-sodium sulfate, 5alpha-Pregnan-3beta-20R-diol 20-sodium sulfate, 5alpha-Pregnan-3beta, 16alpha-diol,20-one 3-sodium sulfate, delta(8,9)-Dehydroestrone 3-sodium sulfate, Estra-3beta, 17alpha-diol 3-sodium sulfate, 3beta-Hydroxy-estr-5(10)-en, 17-one 3-sodium sulfate or 5alpha-Pregnan-3beta,16alpha, 20R-triol 3-sodium sulfate, equol or enterolactone; or a pharmaceutically acceptable salt or ester thereof.

18. A pharmaceutical composition of claim 17 wherein the one or more estrogens are selected from equilin, equilenin, ethinyl estradiol, 17.beta.-estradiol, dihydroequilenin, 17.beta.-dihydroequilenin, menstranol, conjugated estrogens, sulfate esters of estrone, Sodium estrone sulfate, Sodium equilin sulfate, Sodium 17alpha-dihydroequilin sulfate, Sodium 17alpha-estradiol sulfate, Sodium Delta8,9-dehydroestrone sulfate, Sodium equilenin sulfate, Sodium 17beta-dihydroequilin sulfate, Sodium 17alpha-dihydroequilenin sulfate, Sodium 17beta-estradiol sulfate, Sodium 17beta-dihydroequilenin sulfate, Estrone 3-sodium sulfate, Equilin 3-sodium sulfate, 17alpha-Dihydroequilin 3-sodium sulfate, 3beta-Hydroxy-estra-5(10), 7-dien-17-one 3-sodium sulfate, 5alpha-Pregnan-3beta-20R-diol 20-sodium sulfate, 5alpha-Pregnan-3beta, 16alpha-diol,20-one 3-sodium sulfate, delta(8,9)-Dehydroestrone 3-sodium sulfate, Estra-3beta, 17alpha-diol 3-sodium sulfate, 3beta-Hydroxy-estr-5(10)-en, 17-one 3-sodium sulfate or 5alpha-Pregnan-3beta,16alpha, 20R-triol 3-sodium sulfate, equol or enterolactone; or a pharmaceutically acceptable salt or ester thereof.

L2: Entry 64 of 78   File: USPT   Dec 1, 1998

DOCUMENT-IDENTIFIER: US 5843934 A
TITLE: Uses of estrogen compounds for the treatment of disease

Brief Summary Text (4):
Neurodegenerative diseases have a major impact on society. For example,
approximately 3 to 4 million Americans are afflicted with a chronic
neurodegenerative disease known as Alzheimer's disease. Other examples of chronic
neurodegenerative diseases include diabetic peripheral neuropathy, multiple
sclerosis, amyotrophic lateral sclerosis, Huntingdon's disease and Parkinson's
disease. Not all neurodegenerative diseases are chronic. Some acute
neurodegenerative diseases include stroke, schizophrenia, and epilepsy as well as
hypoglycemia and trauma resulting in injury of the brain, peripheral nerves or
spinal cord. There is a need for improved therapeutic agents and methods for
reversing or retarding neuronal damage associated with each of these conditions.

Brief Summary Text (5):
Neurodegenerative diseases and aging are characterized by a wide range of symptoms
which vary in severity and range from individual to individual. For example,
Alzheimer's disease is characterized by symptoms such as depression, aggression,
impairment in short-term memory, impairment in intellectual ability, agitation,
irritability and restlessness. Since estrogen becomes deficient in post-menopausal
women, and since estrogen is believed to affect mood, some studies have been
undertaken to assess the relief of behavioral symptoms associated with Alzheimer's
disease. Unfortunately, those clinical trials that have been performed to establish
the beneficial effect of estrogen on Alzheimer's disease have concluded that no
statistically significant improvements in the disease course or symptoms resulted
from the treatment. Fillet et al. 1986, Psychoneuroendocrinology 11:337-345; Honjo
et al. 1989, Steroid Biochemistry 34:521-524. In one study where only 1 female and
1 male patient were studied and no statistics were available, a rapid reduction in
symptoms of senile dementia was observed when estrogen was administered to the
female patient in a cocktail of drugs together with chorionic gonadotrophin, a
vasodilator and a non-steroidal anti-inflammatory agent after a period as short as
one week (Aroonsakul 1990, U.S. Pat. No. 4,897,389). There is a need for a better
understanding of the underlying process of neurodegeneration such that improved
treatment protocols and effective drugs may be designed that are effective at
treating the disease itself so as to bring about a longterm meaningful reversal of
symptoms.

Drawing Description Text (3):
FIG. 1 shows a histogram of the effects of 17.beta.-estradiol (E.sub.2) on the age
related release of lactate dehydrogenase (LD) in primary cortical neuronal
cultures.

Detailed Description Text (4):
.beta. estrogen is the .beta. isomer of estrogen compounds. .alpha. estrogen is
the .alpha. isomer of estrogen components. The term "estradiol" is either .alpha.
or .beta. estradiol unless specifically identified.

Detailed Description Text (5):
The term "E.sub.2 " is synonymous with .beta.-estradiol 17.beta.-estradiol

and .beta.-E.sub.2. .alpha.E.sub.2, .alpha.-E.sub.2, and .alpha.-estradiol is
the .alpha. isomer of .beta.-E.sub.2 estradiol.

Detailed Description Text (8):
"Neurodegenerative disorder" is defined here and in the claims as a disorder in
which progressive loss of neurons occurs either in the peripheral nervous system or
in the central nervous system. Examples of neurodegenerative disorders include:
chronic neurodegenerative diseases such as Alzheimer's disease, Parkinson's
disease, Huntington's chorea, diabetic peripheral neuropathy, multiple sclerosis,
amyotrophic lateral sclerosis; aging; and acute neurodegenerative disorders
including: stroke, traumatic brain injury, schizophrenia, peripheral nerve damage,
hypoglycemia, spinal cord injury, epilepsy, and anoxia and hypoxia.

Detailed Description Text (12):
In comparison to .beta. estrogen, .alpha. estrogen is typically believed to be at
least 100-1000 times less potent in estrogenic activity. Numerous examples have
been reported in the literature that show that the biological effects of .beta.
estrogen are not shared by the .alpha. isomer. In fact, in the art, .alpha.
estrogen is typically used as a negative control for .beta. estradiol.

Detailed Description Text (16):
These examples demonstrate that both .alpha. and .beta. estradiol at
physiologically relevant doses exert a cytoprotective effect on both glial and
neuroblastoma cells lines in vitro and that this cytoprotective effect can be
distinguished from a mitogenic action. While not wishing to be bound by theory, we
hypothesize that estrogen exerts a direct protective effect on neuronal cells.

Detailed Description Text (17):
In vivo studies described in Examples 3-6 have shown that estrogen can reverse an
impairment in non-spatial learning. This impairment is correlated with a time
dependent decline in choline acetyl transferase (ChAT) in both the frontal cortex
and the hippocampus which is attenuated in animals treated with estradiol. The
ChAT-containing nerve terminals in these two brain regions have cell bodies located
in the basal forebrain. In Example 3, rats have shown improvements in behavioral
performances as determined by the active avoidance test following the addition of
estrogen to estrogen deficient ovariectomized animals. Collectively, these data
provide a method of treating subjects through the modulation of basal forebrain
cholinergic function by means of treating with estrogen so as to reduce loss of
learning and memory associated with neuronal damage.

Detailed Description Text (24):
Biochemical studies on the action of estrogen on cells of the CNS either in vivo or
in vitro has resulted in conflicting reports. A number of studies have shown that
estradiol has an effect on the plasticity of neurons. Morse et al. 1986,
Experimental Neurology 94:649-658, reported that an estrogen derivative enhances
sprouting of commissural-associational afferent fibers in the hippocampal dentate
gyrus following entorhinal cortex lesions. Additionally, cyclic changes in synaptic
density in the CAl of the hippocampus were shown to be related to circulating
E.sub.2 levels (Woolley et al. 1992, Journal of Neuroscience 12:2549-2554) and
these changes could be mimicked with exogenous E.sub.2 administration (Woolley et
al. 1992). Indeed, it has further been shown that ovariectomy reduces and E.sub.2
replacement normalizes high affinity choline uptake (HACU) in the frontal cortex of
rats.

Detailed Description Text (25):
Additionally, Gibbs et al. 1993 (Society for Neuroscience Abstracts 19:5) have
reported upregulation of choline acetyltransferase (ChAT) levels following
estradiol treatment in the medial septum after 2 days and 2 weeks of treatment
although no effect was observed after 1 week using in situ hybridization of CHAT
mRNA. Luine et al. 1980, Brain Research 191:273-277, reported increased ChAT levels

in the preoptic and hypothalamic regions of the rat brain in response to estradiol treatment.

Detailed Description Text (27):
In 1977, Perez-Polo et al. (Life Sci. 21:1535-1543) published a paper entitled "Steroid Induction of Nerve Growth Factor Synthesis in Cell Culture". Although the title indicates a positive effect of steroids on the production of NGF by glial cells in vitro, closer inspection of the paper revealed the opposite. By using non-specific polyclonal antibodies, increased levels of a high molecular weight molecule was identified in response to high levels of estrogen whereas there was no increase in the amount of a low molecular weight (MW) fraction now known to contain .beta. NGF and similarly recognized by the antibody preparation. The doses of estrogen used in the experiment were 5,500 times higher then physiological levels. The levels of the low MW fraction attributed to NGF and secreted into the media were found to be 1000-fold higher than predicted for NGF production using current methods. No further work was carried out by these investigators or others to determine in vivo effects of estrogen on NGF production. Subsequent in vivo studies by Gibbs et al. 1993 reported that steroids (17-.beta. estradiol) caused decreased levels of NGF mRNA in the hippocampus followed by decreased levels in the medial septum and the diagonal band of Broca.

Detailed Description Text (28):
Contrary to the above report, an embodiment of the invention describes how estradiol stimulates the production of neurotrophic growth factor mRNA. For the first time, an estrogen compound has been described that not only has ready access to brain tissue across the blood-brain barrier but can stimulate the availability of growth factor where it is most needed so as to reverse and offset the effects of neurodegeneration.

Detailed Description Text (30):
In a preferred embodiment, both stereoisomers of estradiol, 17-.beta.-estradiol and 17-.alpha.-estradiol have been found effective in reversing neurodegeneration.

Detailed Description Text (31):
In a preferred embodiment, estradiol is administered to rats and also to humans at concentrations sufficient to exert neuroprotective effects in the CNS. These doses vary according to interperson variability, the route of administration and the estrogen formulation used. For example, in rats, estradiol is administered subcutaneously by means of a silastic tube to achieve plasma levels of about 50 pg/ml. In humans, 0.2-10 mg or more specifically 1-2 mg of orally administered estrase (estradiol) given daily is commonly administered to patients suffering from post menopausal syndrome. These levels are also expected to be effective in treating neurodegenerative disorders in human subjects.

Detailed Description Text (34):
The protection of cholinergic neurons from severe degeneration is an important aspect of treatment for patients with acute or chronic neurodegenerative disorders, an example of chronic disease being Alzheimer's disease. For Alzheimer's patients, estrogen replacement or supplementation may be of significant therapeutic use. Other diseases for which estrogen treatment may be effective include Parkinson's disease, Huntington's disease, AIDS Dementia, Wernicke-Korsakoff's related dementia (alcohol induced dementia), age related dementia, age associated memory impairment, brain cell loss due to any of the following; head trauma, stroke, hypoglycemia, ischemia, anoxia, hypoxia, cerebral edema, arteriosclerosis, hematoma and epilepsy; spinal cord cell loss due to any of the conditions listed under brain cell loss; and peripheral neuropathy. Because of its cytoprotective properties, it is suggested that one pathway of action for estrogen is the inhibition of apoptosis.

Detailed Description Text (56):
Results. A single treatment on day 9 with estrogen significantly reduced (p <0.05)

the increase in LD observed in all 6 replicates on days 12 and 16. These data
suggest that in primary neurons, estrogen exposure delays or prevents time-
dependent death in culture for at least 7 days (FIG. 1), an observation that is
further supported by examination of cultures by light microscopy. Here it was
observed that estradiol prevented the retrograde degeneration (regression of
neuronal extensions) and reduced the appearance of cytosolic inclusions (clusters
of material) in cell bodies; both of which are normally observed with aging in
primary neuronal cultures in vitro and with degenerative disorders in vivo.

Detailed Description Text (60):
Results. FIG. 2 shows that hypoglycemia caused a marked and dose-dependent
reduction in both total and live C6 cell numbers in control flasks, which did not
receive E.sub.2 treatment. By contrast, at each of the levels of hypoglycemia
tested, E.sub.2 exposure prevented the loss in total and live cells associated with
hypoglycemia. Trypan blue stains dying cells that have become permeable to the dye.
In adverse conditions, the number of cells in the total cell population that is
measured is diminished as a result of disintegration of dead cells. Hence, the
total cell numbers of cells in samples maintained for 20 hours in suboptimal levels
of glucose show a reduction in total cell number in FIG. 2. However, a large
percentage of this diminishing population are live cells. The addition of estradiol
to cultures, maintained in suboptimal levels of glucose, protects the population
from cell death and results in an overall greater number of live cells. The
asterisk marks those samples having a statistically significant reduction in total
cells and live cells in the absence of estrogen when compared to samples in the
presence of estrogen.

Detailed Description Text (68):
Results. The protective effect of estradiol on the viability of the cell population
is shown in FIG. 3. Pretreatment with estradiol increased the number of live cells
and reduced the number of dead cells in these neuroblastoma cultures following
treatment with NMDA. These data demonstrate that E.sub.2 pretreatment protects
cells from the neurotoxicity associated with excitotoxic amino acids.

Detailed Description Text (71):
The role of estradiol in protecting cholinergic function in the CNS and the
association of this effect with learning and memory has been demonstrated as
described below.

Detailed Description Text (74):
Experimental design. Three groups of animals were analyzed using a standard 2-way
active avoidance paradigm. The three groups of animals were: ovary-intact,
ovariectomized, and estradiol-replaced ovariectomized animals.

Detailed Description Text (76):
Animal surgical procedure: Animals were anesthetized with methoxyflurane (Metofane,
Pitman-Moore, Washington Crossing, N.J.). Two-thirds of the rats underwent
bilateral ovariectomy using a dorsal approach. Three weeks following ovariectomy, a
subset of the ovariectomized animals (the E.sub.2 replaced group) received a 5 mm
Silastic.RTM. (Dow Corning, Midland, Mich.) pellet containing a 1:1 mixture of
cholesterol (Steraloids, Inc., Wilton, N.H.) and 17-B estradiol that was implanted
subcutaneously. Estradiol delivery through Silastic.RTM. tubing results from
diffusion down a large concentration gradient and the fibrosis, which occurs over
time around the Silastic.RTM. pellet, reduces diffusion. The E.sub.2 replacement
regimen was maintained for 2 or 25 weeks following the 3 week rest period after
ovariectomy. At 5 weeks and 28 weeks post ovariectomy, animals were behaviorally
tested. In the long term treatment regimen, we removed and repositioned the
Silastic.RTM. pellets every 2 to 3 weeks to maintain E.sub.2 diffusion from the
Silastic. The ovariectomized group received sham pellets that were similarly re-
positioned every 2 to 3 weeks. Both E.sub.2 and sham pellets were washed twice with
100% ethanol and were then incubated in PBS at room temperature for 48 hours prior

to implantation. The resulting experimental groups were: ovary intact (INTACT), ovariectomized (OVX) (5 or 28 weeks), and estradiol-replaced (E.sub.2 pellet).

Detailed Description Text (85):
Female Sprague-Dawley rats were either ovariectomized (OVX) only, or ovariectomized for 3 weeks followed by subcutaneous implantation of a silastic pellet containing 17-.beta.-estradiol (E.sub.2 pellet) resulting in a replacement of E.sub.2 to physiological levels. Ovary intact animals served as a positive control. Active avoidance behavior and choline acetyltransferase (ChAT) activity in the frontal cortex and hippocampus were assessed at 5 weeks and 28 weeks post ovariectomy while high affinity choline uptake (HACU) was measured only at the 5 week time point.

Detailed Description Text (88):
(a) Plasma estradiol assay. Following behavioral testing, or in the case of the untested animals following the period of treatment, animals were decapitated and trunk blood was obtained. The blood was centrifuged at 13,500.times.g for 1.5 minutes and resulting plasma was aliquoted into a separate tube for estradiol level determination at a later date. Plasma concentrations of E.sub.2 were assayed by radioimmunoassay (RIA) using commercial kits supplied by Diagnostic Product Corp. (Los Angeles, Calif.). The range of assay detectability was 20-3600 pg/ml. All samples were quantified in a single assay.

Detailed Description Text (93):
(a) Estradiol concentrations. Serum E.sub.2 concentrations ere 43.+-.10 and 36.+-.5 pg/ml for the INTACT and E.sub.2 -pellet groups, respectively. Ovariectomy reduced serum E.sub.2 concentrations to below the sensitivity of the radioimmunoassay employed (20 pg/ml) in all but 5 animals sampled. These 5 animals, however, had serum levels that were very close to the sensitivity limit of the assay.

Detailed Description Text (94):
(b) High Affinity Choline Uptake. Ovariectomy significantly reduced HACU by 24% in the frontal cortex (FIG. 6) and by 34% in the hippocampus (FIG. 7). E.sub.2 - replacement resulted in a reversal of this effect of ovariectomy, increasing HACU by 82% in the frontal cortex (FIG. 6) and by 46% in the hippocampus (FIG. 7). Because HACU is a measure of cholinergic activity, it is concluded from these results that estradiol reverses the decline of cholinergic activity in the frontal cortex and hippocampus and further stimulates activity of cholinergic projections to these regions.

Detailed Description Text (97):
In Situ Hybridization Demonstrates Increased Levels of Brain Derived Neurotrophic Factor (BDNF) mRNA is Stimulated by Estradiol.

Detailed Description Text (99):
Results. Ovariectomy resulted in a significant reduction in the BDNF signal in cortical sections relative to INTACT controls. Estradiol replacement of ovariectomized rats increased the BDNF signal to that normally observed in INTACT controls. The data in Table VIII derived multiple slices of the cerebral cortex of an animal in each treatment group demonstrate the stimulatory effect of an estrogen on BDNF synthesis.

Detailed Description Text (101):
In Vivo Studies Demonstrate Increased Levels of NGF mRNA Stimulated by Estradiol.

Detailed Description Text (106):
Adult female rats are ovariectomized and two weeks later are treated with a "Silastic" pellet containing cholesterol (controls) or estradiol in amounts sufficient to elevate plasma estradiol levels in the physiologic range. After 1 to 2 weeks of such estrogen-replacement therapy, rats receive an intracerebral injection of N-methyl-D-aspartate (NMDA) or artificial cerebrospinal fluid in

amounts to induce extensive toxicity of brain neurons.

Detailed Description Text (111):
The effect of estradiol benzoate (E.sub.2 B) on cerebral glucose uptake in various brain regions which contain variable numbers of E.sub.2 B receptors is described and the determination concerning increases in the amount of glucose transported into the brain in the presence of E.sub.2 B is also described for a selected animal model.

Detailed Description Paragraph Table (2):
TABLE II
Commercial Estrogen Preparations

Estrone Aqueous Suspensions Usual dasage IM, 0.1 to 0.5 mg, 2 to 3 times weekly. Estradiol Estrase: 1 to 2 mg PO, daily for three weeks; one week off. Estradiol Conjugates in Oil. a. Estradiol-cypionate 1 to 5 mg IM; every 3 to 4 weeks-more than 20 preparations. b. Estradiol-valerate 10 to 20 mg IM; every 4 weeks - more than 30 preparations. Oral estrogen preparations 50 to 65% estrone sulfate and 20 to 35% equilin sulfate. a. Premarin 0.3 to 2.5 mg PO daily for 3 weeks; one week b. Estrocon 0.625 to 2.5 mg PO daily for 3 weeks; one week off. c. Progens 0.625 to 2.5 mg PO daily for 3 weeks; one week off. d. Many others 0.625 to 2.5 mg PO daily for 3 weeks; one week off. Oral Esterified Estrogens 75 to 85% estrone sulfate and 6 to 15% equilin sulfate. a. Estratab 0.3 to 2.5 mg PO daily for three weeks; one week off. b. Menest 0.3 to 2.5 mg PO daily for three weeks; one week off. Estropipate Piperazine Estrone Sulfate. a. Ogen 0.625 to 5 mg PO daily for 3 weeks: one week off. Ethinyl Estradiol. a. Estinyl 0.02 to 0.5 mg PO daily for three weeks; one week off. b. Feminone 0.05 mg PO daily for three weeks; one week off. Quinestrol a fat stored, slow release form of ethinyl estradiol. a. Estrovis 100 .mu.g PO daily for 7 days; 100, ug weekly thereafter. Diethylstilbestrol (0.2 to 0.5 mg PO daily for three weeks; one week off. 10. Chlorotrianisene Tace; 12 to 25 mg PO daily for three weeks; on week off. Oral Estrogen-Antianxiety Agent Combinations Oral. a. Milprem-200 or -400 (Conjugated estrogens and meprobamate). b. PMB 20 or 400 (Conjugated estrogens and meprobamate). c. Menrium 5-2, 5-4, or 10-4 esterified estrogens and chlordiazepoxide. Estrogen and Androgen Combinations IM in Oil. a. 13 preparations with 2 mg estradiol cypionate and 50 mg testosterone cypionate. b. 12 preparations with 4 mg estradiol valerate and 90 mg testosterone enanthate. c. 4 preparations with 8 mg estradiol valerate and 180 mg testosterone enanthate. d. 6 preparations with various combinations of other estrogens and androgens.

Detailed Description Paragraph Table (5):
TABLE V   Effect of Short-Term and Long-Term Ovariectomy and Estradiol Replacement on Learning and Retention Days to Reach Criteria 5 weeks 28 weeks Treatment Group (acute) (chronic)
INTACT 14.0 .+-. 9.0 9.0 .+-. 2.8
OVARIECTOMIZED 15.0 .+-. 0.0 15.0 .+-. 0.0 E.sub.2 PELLET .sup. 9.5 .+-. 2.1.sup.# 1.3 .+-. 0.3* .sup.# p .ltoreq. 0.05 vs Ovariectomized and Intact animals. *p .ltoreq. 0.05 vs Ovariectomized animals using the MannWhitney U nonparametric statistic.

Detailed Description Paragraph Table (6):
TABLE VI   Effect of Short Term and Long Term Ovariectomy and Estradiol Replacement on Choline Acetyltransferase Activity in the Frontal Cortex ChAT activity (nmol/30 min/mg protein) Treatment Group 5 weeks 26 weeks   INTACT 10.2 + 0.5 4.0 + 0.1
OVARIECTOMIZED 9.2 + 0.6 4.0 + 0.2 E.sub.2 PELLET 9.8 + 0.6 8.2 + 0.8*
n = 6 for ovariectomized and E.sub.2 pellet groups for the 5 week time period. n = 5 S for intact group for the 5 week time period. n = 6 for all treatment groups for the 28 week time period. p .ltoreq. 0.05

vs intact group and OVX group.

Detailed Description Paragraph Table (7):
TABLE VII _____ Effect of Short Term and Long Term
Ovariectomy and Estradiol Replacement on Choline Acetyltransferase Activity in the
Hippocampus. ChAT activity (nmol/30 min/mg protein) Treatment Group 5 weeks 28
weeks _____ INTACT 13.2 + 0.8 5.7 + 0.3
OVARIECTOMTZED 10.3 .+-. 0.3* 6.2 .+-. 1.1 E.sub.2 PELLET 12.7 .+-. 0.5 8.0 .+-.
1.1 _____ n = 6 for ovariectomized and E.sub.2
pellet groups for the 5 week time period. n = 5 for intact group for the 5 week
time period. n = 6 for all treatment groups for the 28 week time period.
*p .ltoreq. 0.05 vs. intact group and E.sub.2 pellet group.

CLAIMS:

5. A method according to claim 3, wherein the estrogen compound is alpha estradiol.

6. A method according to claim 4, wherein the estrogen compound is alpha estradiol.

L2: Entry 75 of 78   File: USPT   Sep 10, 1996

DOCUMENT-IDENTIFIER: US 5554601 A
** See image for Certificate of Correction **
TITLE: Methods for neuroprotection

Abstract Text (1):
A method is provided for conferring neuroprotection on a population of cells using
estrogen compounds that have insubstantial sex activity and furthermore, a method
is provided that utilizes estrogen compounds in the absence of testosterone for
treating neurodegenerative diseases including Alzheimer's disease so as to retard
the adverse effects of these disorders, Examples of estrogen compounds that have
insubstantial sex activity includes alpha isomers of estrogen compounds such as
17.alpha. estradiol.

Brief Summary Text (4):
Neurodegenerative diseases have a major impact on society. For example,
approximately 3 to 4 million Americans are afflicted with a chronic
neurodegenerative disease known as Alzheimer's disease. Other examples of chronic
neurodegenerative diseases include diabetic peripheral neuropathy, multiple
sclerosis, amyotrophic lateral sclerosis, Huntingdon's disease and Parkinson's
disease. Not all neurodegenerative diseases are chronic. Some acute
neurodegenerative diseases include stroke, schizophrenia, and epilepsy as well as
hypoglycemia and trauma resulting in injury of the brain, peripheral nerves or
spinal cord. There is a need for improved therapeutic agents and methods for
reversing or retarding neuronal damage associated with each of these conditions.

Brief Summary Text (5):
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which vary in severity and range from individual to individual. For example,
Alzheimer's disease is characterized by symptoms such as depression, aggression,
impairment in short-term memory, impairment in intellectual ability, agitation,
irritability and restlessness. Since estrogen becomes deficient in post-menopausal
women, and since estrogen is believed to affect mood, some studies have been
undertaken to assess the relief of behavioral symptoms associated with Alzheimer's
disease. Unfortunately, those clinical trials that have been performed to establish
the beneficial effect of estrogen on Alzheimer's disease have concluded that no
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female patient in a cocktail of drugs together with chorionic gonadotrophin, a
vasodilator and a non-steroidal anti-inflammatory agent after a period as short as
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related release of lactate dehydrogenase (LD) in primary cortical neuronal
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.beta. estrogen is the .beta. isomer of estrogen compounds. .alpha. estrogen is
the .alpha. isomer of estrogen components. The term "estradiol" is either .alpha.
or .beta. estradiol unless specifically identified.

Detailed Description Text (5):
The term "E.sub.2 " is synonymous with .beta.-estradiol 17.beta.-estradiol
and .beta.-E.sub.2. .alpha.E.sub.2, .alpha.-E.sub.2, and .alpha.-estradiol is
the .alpha. isomer of .beta.-E.sub.2 estradiol.

Detailed Description Text (8):
"Neurodegenerative disorder" is defined here and in the claims as a disorder in
which progressive loss of neurons occurs either in the peripheral nervous system or
in the central nervous system. Examples of neurodegenerative disorders include:
chronic neurodegenerative diseases such as Alzheimer's disease, Parkinson's
disease, Huntington's chorea, diabetic peripheral neuropathy, multiple sclerosis,
amyotrophic lateral sclerosis; aging; and acute neurodegenerative disorders
including: stroke, traumatic brain injury, schizophrenia, peripheral nerve damage,
hypoglycemia, spinal cord injury, epilepsy, and anoxia and hypoxia.

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These examples demonstrate that both .alpha. and .beta. estradiol at
physiologically relevant doses exert a cytoprotective effect on both glial and
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hypothesize that estrogen exerts a direct protective effect on neuronal cells.

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in the basal forebrain. In Example 3, rats have shown improvements in behavioral
performances as determined by the active avoidance test following the addition of
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sprouting of commissural-associational afferent fibers in the hippocampal dentate
gyrus following entorhinal cortex lesions. Additionally, cyclic changes in synaptic
density in the CA1 of the hippocampus were shown to be related to circulating
E.sub.2 levels (Woolley et al. 1992, Journal of Neuroscience 12:2549-2554) and
these changes could be mimicked with exogenous E.sub.2 administration (Woolley et

al. 1992). Indeed, it has further been shown that ovariectomy reduces and E.sub.2 replacement normalizes high affinity choline uptake (HACU) in the frontal cortex of rats.

Detailed Description Text (25):
Additionally, Gibbs et al. 1993 (Society for Neuroscience Abstracts 19:5) have reported upregulation of choline acetyltransferase (ChAT) levels following estradiol treatment in the medial septum after 2 days and 2 weeks of treatment although no effect was observed after 1 week using in situ hybridization of ChAT mRNA. Luine et al. 1980, Brain Research 191:273-277, reported increased ChAT levels in the preoptic and hypothalamic regions of the rat brain in response to estradiol treatment.

Detailed Description Text (27):
In 1977, Perez-Polo et al. (Life Sci. 21:1535-1543) published a paper entitled "Steroid Induction of Nerve Growth Factor Synthesis in Cell Culture". Although the title indicates a positive effect of steroids on the production of NGF by glial cells in vitro, closer inspection of the paper revealed the opposite. By using non-specific polyclonal antibodies, increased levels of a high molecular weight molecule was identified in response to high levels of estrogen whereas there was no increase in the amount of a low molecular weight (MW) fraction now known to contain .beta. NGF and similarly recognized by the antibody preparation. The doses of estrogen used in the experiment were 5,500 times higher then physiological levels. The levels of the low MW fraction attributed to NGF and secreted into the media were found to be 1000-fold higher than predicted for NGF production using current methods. No further work was carried out by these investigators or others to determine in vivo effects of estrogen on NGF production. Subsequent in vivo studies by Gibbs et al. 1993 reported that steroids (17-.beta. estradiol) caused decreased levels of NGF mRNA in the hippocampus followed by decreased levels in the medial septum and the diagonal band of Broca.

Detailed Description Text (28):
Contrary to the above report, an embodiment of the invention describes how estradiol stimulates the production of neurotrophic growth factor mRNA. For the first time, an estrogen compound has been described that not only has ready access to brain tissue across the blood-brain barrier but can stimulate the availability of growth factor where it is most needed so as to reverse and offset the effects of neurodegeneration.

Detailed Description Text (30):
In a preferred embodiment, both stereoisomers of estradiol, 17-.beta.-estradiol and 17-.alpha.-estradiol have been found effective in reversing neurodegeneration.

Detailed Description Text (31):
In a preferred embodiment, estradiol is administered to rats and also to humans at concentrations sufficient to exert neuroprotective effects in the CNS. These doses vary according to interperson variability, the route of administration and the estrogen formulation used. For example, in rats, estradiol is administered subcutaneously by means of a silastic tube to achieve plasma levels of about 50 pg/ml. In humans, 0.2-10 mg or more specifically 1-2 mg of orally administered estrase (estradiol) given daily is commonly administered to patients suffering from post menopausal syndrome. These levels are also expected to be effective in treating neurodegenerative disorders in human subjects.

Detailed Description Text (34):
The protection of cholinergic neurons from severe degeneration is an important aspect of treatment for patients with acute or chronic neurodegenerative disorders, an example of chronic disease being Alzheimer's disease. For Alzheimer's patients, estrogen replacement or supplementation may be of significant therapeutic use. Other diseases for which estrogen treatment may be effective include Parkinson's

disease, Huntington's disease, AIDS Dementia, Wernicke-Korsakoff's related-dementia (alcohol induced dementia), age related dementia, age associated memory impairment, brain cell loss due to any of the following; head trauma, stroke, hypoglycemia, ischemia, anoxia, hypoxia, cerebral edema, arteriosclerosis, hematoma and epilepsy; spinal cord cell loss due to any of the conditions listed under brain cell loss; and peripheral neuropathy. Because of its cytoprotective properties, it is suggested that one pathway of action for estrogen is the inhibition of apoptosis.

Detailed Description Text (53):
Results. A single treatment on day 9 with estrogen significantly reduced (p<0.05) the increase in LD observed in all 6 replicates on days 12 and 16. These data suggest that in primary neurons, estrogen exposure delays or prevents time-dependent death in culture for at least 7 days (FIG. 1), an observation that is further supported by examination of cultures by light microscopy. Here it was observed that estradiol prevented the retrograde degeneration (regression of neuronal extensions) and reduced the appearance of cytosolic inclusions (clusters of material) in cell bodies; both of which are normally observed with aging in primary neuronal cultures in vitro and with degenerative disorders in vivo.

Detailed Description Text (56):
Results. FIG. 2 shows that hypoglycemia caused a marked and dose-dependent reduction in both total and live C6 cell numbers in control flasks, which did not receive E.sub.2 treatment. By contrast, at each of the levels of hypoglycemia tested, E.sub.2 exposure prevented the loss in total and live cells associated with hypoglycemia. Trypan blue stains dying cells that have become permeable to the dye. In adverse conditions, the number of cells in the total cell population that is measured is diminished as a result of disintegration of dead cells. Hence, the total cell numbers of cells in samples maintained for 20 hours in suboptimal levels of glucose show a reduction in total cell number in FIG. 2. However, a large percentage of this diminishing population are live cells. The addition of estradiol to cultures, maintained in suboptimal levels of glucose, protects the population from cell death and results in an overall greater number of live cells. The asterisk marks those samples having a statistically significant reduction in total cells and live cells in the absence of estrogen when compared to samples in the presence of estrogen.

Detailed Description Text (62):
Results. The protective effect of estradiol on the viability of the cell population is shown in FIG. 3. Pretreatment with estradiol increased the number of live cells and reduced the number of dead cells in these neuroblastoma cultures following treatment with NMDA. These data demonstrate that E.sub.2 pretreatment protects cells from the neurotoxicity associated with excitotoxic amino acids.

Detailed Description Text (64):
The role of estradiol in protecting cholinergic function in the CNS and the association of this effect with learning and memory has been demonstrated as described below.

Detailed Description Text (66):
Experimental design. Three groups of animals were analyzed using a standard 2-way active avoidance paradigm. The three groups of animals were: ovary-intact, ovariectomized, and estradiol-replaced ovariectomized animals.

Detailed Description Text (68):
Animal surgical procedure: Animals were anesthetized with methoxyflurane (Metofane, Pitman-Moore, Washington Crossing, N.J.). Two-thirds of the rats underwent bilateral ovariectomy using a dorsal approach. Three weeks following ovariectomy, a subset of the ovariectomized animals (the E.sub.2 replaced group) received a 5 mm Silastic.RTM. (Dow Corning, Midland, Mich.) pellet containing a 1:1 mixture of cholesterol (Steraloids, Inc., Wilton, N.H.) and 17-B estradiol that was implanted

subcutaneously. Estradiol delivery through Silastic.RTM. tubing results from diffusion down a large concentration gradient and the fibrosis, which occurs over time around the Silastic.RTM. pellet, reduces diffusion. The E.sub.2 replacement regimen was maintained for 2 or 25 weeks following the 3 week rest period after ovariectomy. At 5 weeks and 28 weeks post ovariectomy, animals were behaviorally tested. In the long term treatment regimen, we removed and repositioned the Silastic.RTM.pellets every 2 to 3 weeks to maintain E.sub.2 diffusion from the Silastic. The ovariectomized group received sham pellets that were similarly re-positioned every 2 to 3 weeks. Both E.sub.2 and sham pellets were washed twice with 100% ethanol and were then incubated in PBS at room temperature for 48 hours prior to implantation. The resulting experimental groups were: ovary intact (INTACT), ovariectomized (OVX) (5 or 28 weeks), and estradiol-replaced (E.sub.2 pellet).

Detailed Description Text (76):
Female Sprague-Dawley rats were either ovariectomized (OVX) only, or ovariectomized for 3 weeks followed by subcutaneous implantation of a silastic pellet containing 17-.beta.-estradiol (E.sub.2 pellet) resulting in a replacement of E.sub.2 to physiological levels. Ovary intact animals served as a positive control. Active avoidance behavior and choline acetyltransferase (ChAT) activity in the frontal cortex and hippocampus were assessed at 5 weeks and 28 weeks post ovariectomy while high affinity choline uptake (HACU) was measured only at the 5 week time point.

Detailed Description Text (79):
(a) Plasma estradiol assay. Following behavioral testing, or in the case of the untested animals following the period of treatment, animals were decapitated and trunk blood was obtained. The blood was centrifuged at 13,500.times.g for 1.5 minutes and resulting plasma was aliquoted into a separate tube for estradiol level determination at a later date. Plasma concentrations of E.sub.2 were assayed by radioimmunoassay (RIA) using commercial kits supplied by Diagnostic Product Corp. (Los Angeles, Calif.). The range of assay detectability was 20-3600 pg/ml. All samples were quantified in a single assay.

Detailed Description Text (84):
(a) Estradiol concentrations. Serum E.sub.2 concentrations were 43.+-.10 and 36.+-.5 pg/ml for the INTACT and E.sub.2 -pellet groups, respectively. Ovariectomy reduced serum E.sub.2 concentrations to below the sensitivity of the radioimmunoassay employed (20 pg/ml) in all but 5 animals sampled. These 5 animals, however, had serum levels that were very close to the sensitivity limit of the assay.

Detailed Description Text (85):
(b) High Affinity Choline Uptake. Ovariectomy significantly reduced HACU by 24% in the frontal cortex (FIG. 6) and by 34% in the hippocampus (FIG. 7). E.sub.2 -replacement resulted in a reversal of this effect of ovariectomy, increasing HACU by 82% in the frontal cortex (FIG. 6) and by 46% in the hippocampus (FIG. 7). Because HACU is a measure of cholinergic activity, it is concluded from these results that estradiol reverses the decline of cholinergic activity in the frontal cortex and hippocampus and further stimulates activity of cholinergic projections to these regions.

Detailed Description Text (87):
Example 4a: In situ Hybridization Demonstrates Increased Levels of Brain Derived Neurotrophic Factor (BDNF) mRNA is Stimulated by Estradiol.

Detailed Description Text (90):
Results. Ovariectomy resulted in a significant reduction in the BDNF signal in cortical sections relative to INTACT controls. Estradiol replacement of ovariectomized rats increased the BDNF signal to that normally observed in INTACT controls. The data in Table VIII derived multiple slices of the cerebral cortex of an animal in each treatment group demonstrate the stimulatory effect of an estrogen

on BDNF synthesis.

Detailed Description Text (91):
Example 4b: In Vivo Studies Demonstrate Increased Levels of NGF mRNA Stimulated by Estradiol.

Detailed Description Text (95):
Adult female rats are ovariectomized and two weeks later are treated with a "Silastic" pellet containing cholesterol (controls) or estradiol in amounts sufficient to elevate plasma estradiol levels in the physiologic range. After 1 to 2 weeks of such estrogen-replacement therapy, rats receive an intracerebral injection of N-methyl-D-aspartate (NMDA) or artificial cerebrospinal fluid in amounts to induce extensive toxicity of brain neurons.

Detailed Description Text (99):
The effect of estradiol benzoate (E.sub.2 B) on cerebral glucose uptake in various brain regions which contain variable numbers of E.sub.2 B receptors is described and the determination concerning increases in the amount of glucose transported into the brain in the presence of E.sub.2 B is also described for a selected animal model.

Detailed Description Paragraph Table (2):
TABLE II
Commercial Estrogen Preparations

Estrone
Aqueous Suspensions Usual dosage IM, 0.1 to 0.5 mg, 2 to 3 times weekly. Estradiol Estrase: 1 to 2 mg PO, daily for three weeks; one week off. Estradiol Conjugates in Oil. a. Estradiol-cypionate 1 to 5 mg IM; every 3 to 4 weeks-more than 20 preparations. b. Estradiol-vaterate 10 to 20 mg IM; every 4 weeks - more than 30 preparations. Oral estrogen preparations 50 to 65% estrone sulfate and 20 to 35% equilin sulfate. a. Premarin 0.3 to 2.5 mg PO daily for 3 weeks; one week off. b. Estrocon 0.625 to 2.5 mg PO daily for 3 weeks; one week off. c. Progens 0.625 to 2.5 mg PO daily for 3 weeks; one week off. d. Many others 0.625 to 2.5 mg PO daily for 3 weeks; one week off. Oral Esterified Estrogens 75 to 85% estrone sulfate and 6 to 15% equilin sulfate. a. Estratab 0.3 to 2.5 mg PO daily for three weeks; one week off. b. Menest 0.3 to 2.5 mg PO daily for three weeks; one week off. Estropipate Piperazine Estrone Sulfate. a. Ogen 0.625 to 5 mg PO daily for 3 weeks: one week off. Ethinyl Estradiol. a. Estinyl 0.02 to 0.5 mg PO daily for three weeks; one week off. b. Feminone 0.05 mg PO daily for three weeks; one week off. Quinestrol a fat stored, slow release form of ethinyl estradiol. a. Estrovis 100 .mu.g PO daily for 7 days; 100 .mu.g weekly thereafter. Diethytstilbestrol (0.2 to 0.5 mg PO daily for three weeks; one week off. 10. Chlorotrianisene Tace; 12 to 25 mg PO daily for three weeks; on week off. Oral Estrogen-Antianxiety Agent Combinations Oral. a. Milprem-200 or -400 (Conjugated estrogens and meprobamate). b. PMB 20 or 400 (Conjugated estrogens and meprobamate). c. Menriun 5-2, 5-4, or 10-4 esterified estrogens and chlordiazepoxide. Estrogen and Androgen Combinations IM in Oil. a. 13 preparations with 2 mg estradiol cypionate and 50 mg testosterone cypionate. b. 12 preparations with 4 mg estradiol valerate and 90 mg testosterone enanthate. c. 4 preparations with 8 mg estradiol valerate and 180 mg testosterone enanthate. d. 6 preparations with various combinations of other estrogens and androgens.

Detailed Description Paragraph Table (5):
TABLE V   Effect of Short-Term and Long-Term Ovariectomy and Estradiol Replacement on Learning and Retention Days to Reach Criteria 5 weeks 28 weeks Treatment Group (acute) (chronic)
INTACT 14.0 .+-. 9.0 9.0 .+-. 2.8
OVARIECTOMIZED 15.0 .+-. 0.0 15.0 .+-. 0.0 E.sub.2 PELLET 9.5 .+-. 2.1.sup.#
1.3 .+-. 0.3*   .sup.# p .ltoreq. 0.05 vs

Ovariectomized and Intact animals. *p .ltoreq. 0.05 vs Ovariectomized animals using the MannWhitney U nonparametric statistic.

Detailed Description Paragraph Table (6):
TABLE VI _____ Effect of Short Term and Long Term Ovariectomy and Estradiol Replacement on Choline Acetyltransferase Activity in the Frontal Cortex ChAT activity (nmol/30 min/mg protein) Treatment Group 5 weeks 28 weeks _____ INTACT 10.2 .+-. 0.5 4.0 .+-. 0.1 OVARIECTOMIZED 9.2 .+-. 0.6 4.0 .+-. 0.2 E.sub.2 PELLET 9.8 .+-. 0.6 8.2 .+-. 0.8* _____ n = 6 for ovariectomized and E.sub.2 pellet groups for the 5 week time period. n = 5 S for intact group for the 5 week time period. n = 6 for all treatment groups for the 28 week time period. *p .ltoreq. 0.05 vs intact group and OVX group.

Detailed Description Paragraph Table (7):
TABLE VII _____ Effect of Short Term and Long Term Ovariectomy and Estradiol Replacement on Choline Acetyltransferase Activity in the Hippocampus. ChAT activity (nmol/30 min/mg protein) Treatment Group 5 weeks 28 weeks _____ INTACT 13.2 .+-. 0.8 5.7 .+-. 0.3 OVARIECTOMIZED 10.3 .+-. 0.3* 6.2 .+-. 1.1 E.sub.2 PELLET 12.7 .+-. 0.5 8.0 .+-. 1.1 _____ n = 6 for ovariectomized and E.sub.2 pellet groups for the 5 week time period. n = 5 for intact group for the 5 week time period. n = 6 for all treatment groups for the 28 week time period. *p < 0.05 vs. intact group and E.sub.2 pellet group.

CLAIMS:

24. A method according to claim 23, wherein the estrogen compound is .alpha.-estradiol.

26. A method according to claim 23, wherein the neurodegenerative disorder is Alzheimer's disease and the effective dose of the estrogen compound provides protection of a population of nerve cells from progressive cell damage leading to cell death otherwise occurring without any intervention.

27. A method according to claim 25, wherein the neurodegenerative disorder is Alzheimer's disease and the effective dose of estrogen compound provides protection of a population of nerve cells from progressive cell damage leading to cell death otherwise occurring without any intervention.

L2: Entry 76 of 78   File: USPT   Aug 27, 1996

DOCUMENT-IDENTIFIER: US 5550029 A
TITLE: Method for diagnosing estrogen responsiveness

Brief Summary Text (4):
One of the most prevalent forms of hormone therapy today is that of estrogen
replacement therapy in women. Estrogen therapy is prescribed widely not only for
the alleviation of menopausal symptoms but also for the prevention of osteoporosis
and cardiovascular disease. Furthermore, estrogen may have utility as a therapeutic
treatment for chronic neurodegenerative diseases such as Alzheimer's disease.

Brief Summary Text (6):
Numerous studies on the treatment of menopause with estrogen have revealed a
dramatic biological variability in response to a common event--the 95% or greater
decline in ovarian estradiol (E.sub.2 .beta.) at the menopause. The interpersonal
variability in symptoms that is a hallmark of the decline in estrogen levels during
menopause is also manifest in the response of individuals to estrogen replacement
therapy. Seventy five to 80% of women undergoing the natural menopause, and 95-100%
of oophorectomized women experience physiological and/or psychological problems
associated with the decline of steroids (Utian (1977), Obstet. Gynecol. Survey
32:193-97). Twenty to 25% of women are unaffected (Haenngi et al. (1993), Maturitas
16:111-122), the remainder of women have symptoms that exhibit a wide variance.
These symptoms include hot flushes, perspiration, muscle and joint pain, fatigue,
headaches and irritability and vary greatly in both their intensity and frequency
(Utian (1977)). Hot flushes, the cardinal sign of the menopause, persist for more
than one year in 95% of affected women (Jaszmann et al. (1969), Med. Gynecol.
Sociol. 4:268-277; McKinlay et al. (1974), Br. J. Prev. Soc. Med. 28:108-111). Some
women experience as few as one flush per month while others have one flush per hour
(Jaszman et al. (1969), McKinley et al. (1974)). Menopausal hot flushes can also
vary in severity and/or impact from not bothersome to totally incapacitating--in
some cases preventing afflicted women from working. Treatment of these menopausal
symptoms by estrogen replacement therapy has proved to be effective for some
individuals and not for others.

Detailed Description Text (4):
The term "E.sub.2 .beta." as used in this description and in the claims means
17.beta.-estradiol and is an example of an "estrogen compound" here defined as a
form of estrogen including derivative or analogue, either steroidal or non-
steroidal that has an effect in this assay.

Detailed Description Text (37):
Freshly drawn heparinized samples (2.5 ml blood with 250 units heparin sodium in
sterile water; sodium heparin from Elkins-Sinn, Inc., Cherry Hill, N.J.) were
placed in 15 ml Corning polypropylene sterile centrifuge tubes with plugged seal
caps (Corning Glass Works, Corning, N.Y.). The cells were immediately isolated from
the plasma by centrifugation at 1500 rpms (416.times.Gs) for 20 minutes at
4.degree. C. After the first centrifugation, the buffy coat containing the white
blood cells was removed and the serum samples were frozen at -90.degree. C. for
estradiol (E.sub.2 .beta.) assay. Also at this time, an aliquot of serum was
assayed for glucose and lactate (YSI Stat 2300 Analyzer, YSI, Inc., Yellow Springs,
Ohio). The RBC fraction was washed three times and precipitated by centrifugation

at 4.degree. C. and 1500 rpm for 20 minutes in between washes. This paradigm has been demonstrated to reduce intracellular levels of glucose to very low levels (Gasbjerg et al. (1990), Biochem. et Biophys. Acta 1062:83-93). The wash buffer was 37.degree. C. Krebs Ringer Phosphate (glucose free) consisting of 136 mM NaCl, 5 mM NaH.sub.2 PO.sub.4, 1 mM CaCl.sub.2, 1.25 mM MgSO.sub.4, 4.7 mM KCl and supplemented with 200 mM 1-glutamine, pH 7.4. Finally, the cells were counted on a hemacytometer and resuspended in one of the following buffers to obtain a concentration of 5 million cells/ml:

Detailed Description Text (39):
(2) Krebs' Ringer Phosphate+hormone (estradiol (E.sub.2 .beta.) (544 pg/ml)).

Detailed Description Text (48):
FIG. 1 depicts the typical values obtained from RBCs when they are exposed in vitro to estradiol (E.sub.2 .beta.) or to control media (controls). Of the 21 patients examined, 14 showed a significant response to in vitro exposure to E.sub.2 .beta., while 7 patients failed to respond to the estrogen.

Detailed Description Text (50):
Of the females, 9 of 12 subjects responded to E.sub.2 .beta. with a mean response of 230% (Table III, FIG. 2). Three of 12 female subjects failed to respond to the E.sub.2 .beta. exposure and exhibited only a 39% increase in C.sup.14 -2-DG uptake after 4 hours of exposure to estradiol (Table III). Male subjects were similarly analyzed for the significance of their response to E.sub.2 .beta.. Five of 9 males tested showed a significant response to the steroid, while 4 subjects failed to respond (FIG. 3). The 5 responders showed a 188% increase in C.sup.14 -2-DG uptake in response to E.sub.2 .beta., while the nonresponders showed a -1% response to the steroid (Table III). Interestingly, the lack of response of the nonresponder males appeared to be related to the high initial values of C.sup.14 -2-DG uptake (FIG. 3).

Detailed Description Text (52):
Patient G was a 53 year old female who required estrogen replacement postmenopausally. She suffered from depression and hot flushes when not on estrogen and was taking Premarin at the time of the blood sample.

Detailed Description Text (54):
As can be seen from FIG. 1, patient G had a relatively high level of responsiveness to estradiol whereas patient K had a small but lower level of responsiveness. Based on the predictive results of the test, patient G would have been recommended to use an estrogen replacement therapy whereas it is likely that patient K would not have been so advised.

Detailed Description Text (59):
(c) Correlation of questionnaire results detailing biological parameters characteristic of each patient with in vitro results. We reviewed the impact of questionnaire results on the magnitude of the in vitro C.sup.14 -2-DG uptake response of RBCs to E.sub.2 .beta. exposure. As shown in Table IV, when the Spearman's Rank Correlation Statistical Test was applied to the data, no correlations were found. As such, it appears that a variety of factors which might be expected to influence glucose uptake, such as age, body weight, stage of the menstrual cycle, plasma estradiol or glucose concentrations do not influence this in vitro response. Indeed, we have observed, as described above and depicted in FIG. 4, that time of day and recent food intake do not effect the in vitro response of RBCs to E.sub.2 .beta.. As such, the test of estrogen responsiveness appears to be robust and resistant to compromising factors.

Detailed Description Text (65):
As depicted in Table V, four of seven subjects showed a significant increase in .sup.14 C-2-DG deoxyglucose uptake into RBCs in response to in vitro exposure to

estradiol. In the three non-responders, the % response to estradiol was 98.9.+-.5.5% (100%=uptake in control samples from the same subjects). By contrast, the 4 responders showed an estradiol effect on .sup.14 C-2-DG deoxyglucose uptake of 164.+-.19.4% (p<0.05 vs. response in the non-responders).

Detailed Description Text (67):
The data shows good agreement between the in vitro response of RBCs to estradiol and the symptomology of the subjects. While not wishing to be bound by theory, we propose that based on the results obtained in this study, symptomatic subjects may exhibit an estrogen-dependence of their GLUT1 transporter mechanism and as such require estrogen for the sufficient transport of glucose into the brain. By contrast, the non-symptomatic subjects may have an estrogen-independent GLUT1 transport and therefore do not require the ovarian hormone to maintain sufficient delivery of glucose to the brain.

Detailed Description Text (90):
The effect of estradiol benzoate (E.sub.2 .beta.) on cerebral glucose uptake in various brain regions which contain variable numbers of E.sub.2 .beta. receptors is described and the determination concerning increases in the amount of glucose transported into the brain in the presence of E.sub.2 .beta. is also described for a selected animal model.

Detailed Description Paragraph Table (5):
TABLE V
EFFECTS OF IN VITRO EXPOSURE OF RBCs TO E.sub.2 .beta. ON .sup.14 C-2- DEOXYGLUCOSE UPTAKE IN SEVEN POST-MENOPAUSAL WOMEN Subject Subject Subject Subject Subject Subject Subject 1 2 3 4 5 6 7
%
Response 85 .+-. 2 103 .+-. 3 154 .+-. 8* 137 .+-. 6* 108 .+-. 5 135 .+-. 24* 230 .+-. 40* to Estradiol # Flushes 0 0.067 2 5.5 0 0.05 12 per day Vaginal 0 0 3 10 N/A 0 8 Dryness Index Sleep 0 0 5 5 0 5 4 Disturbance Emotionality 0 0 1 5 0 0 5 Composite 0 0.067 8 15.5 0 5.05 21 Neurological Score
%
Response to Estradiol: Depicts the dpm of .sup.14 C2-DG taken up into 4 to 5 replicates of RBCs exposed to E.sub.2 .beta. divided by dpms .sup.14 C2-DG taken up into 4 to 5 replicates of RBCs exposed to control media multiplied by 100. # Hot Flushes Per Day: Was determined from a survey conducted at the time of the blood sampling. Vaginal Dryness Index: Was determined from the number of painful intercourses of the last 10 occurrences. Sleep Disturbance: Subjects were asked to rate the extent of their proble with spontaneous awakening during the night (on a 1 to 5 scale, with 5 being the worst). Emotionality: Was scored based upon the subjective assessment of unexplaned crying episodes (on a 1 to 5 scale, with 5 being the worst). Composite Neurological Score: Number of hot flushes per day plus subjective rating of sleep disturbances plus subjective rating of emotionality. *Indicates a significant response to E.sub.2 .beta. (p < 0.05) as analyze by a 2way ttest for independent samples of 4 to 5 samples in each of the control and E2 exposed cells.